Increased Calcium Sensitivity of Cardiomyocyte Contractions Results in Cardiomyopathy and Arrhythmia

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HYPERTROPHIC CARDIOMYOPATHY (HCM) is characterized by cardiac ventricular hypertrophy with diastolic dysfunction. More than half of HCM patients have a family history of HCM and/or sudden cardiac death consistent with autosomal dominant inheritance. Because the prevalence of this disease in the general population is 1 in 500, HCM is one of the most prevalent hereditary diseases.

HCM is a major cause of sudden death in young and progressive heart failure as well as arrhythmia in adults. In such cases, cardiac transplantation may be required for severe cases. Pathologically, cardiomyocyte hypertrophy and myofibrillar disarray are found in the heart. Recent studies have revealed that mutations in genes encoding the components of sarcomere cause HCM, which is often associated with the increased Ca^{2+} sensitivity of muscle contractions. However, it is not known whether abnormal Ca^{2+} sensitivity would directly result in clinical and pathological phenotypes of HCM.

A sarcomere is a fundamental unit of muscle structure, composed of actin-based thin filaments and myosin-based thick filaments. Muscle contractions are regulated by intracellular Ca^{2+}. The Ca^{2+} sensitivity of cardiac muscle contractions is regulated by two different mechanisms: (1) regulation by troponin complex acting on the thin filament and (2) regulation by myosin light chain acting on the thick filament.

We have previously shown that a heart-specific myosin light chain phosphatase small subunit, HS-M_{21}, increases the Ca^{2+}-sensitivity of cardiac muscle contraction. In this study, we investigated the function of HS-M_{21} *in vivo* and the causative role of abnormal Ca^{2+} sensitivity in HCM. We generated transgenic (Tg) mice, in which human HS-M_{21} was specifically expressed in the mouse's heart. Three different Tg (one low-expression line and two high-expression lines) were generated. Increased Ca^{2+} sensitivity of cardiac muscle contraction was confirmed in the two high-expression lines. Although Tg mice with the low-expression line did not show any phenotypes, Tg mice in both high-expression lines developed severe systolic dysfunction with myocardial fibrosis, which resembles the progressive phase of HCM phenotypes. Most notably, the contractile dysfunction and cardiac fibrosis were improved by treatment with the Rho-kinase inhibitor, Fasudil.

Gene expression analysis of Tg mice hearts revealed that so-called cardiac remodeling genes were highly induced even in the low-expression line-, showing no cardiac phenotype and that several key modulators were induced only in the high-expression lines before manifesting with cardiac hypertrophy. In addition, sinus bradycardia and atrioventricular conduction defects were observed in Tg mice with high-expression lines. Our findings showed that increased Ca^{2+} sensitivity of cardiac muscle contractions could directly result in the HCM phenotype, which can be prevented by modulating Rho-kinase activity. These Tg mice may be useful for developing novel therapeutics for HCM.

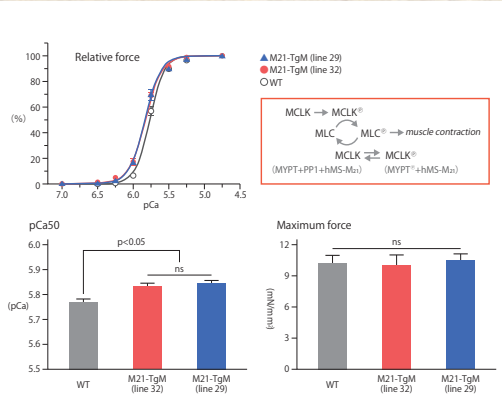

Fig.1: **Calcium sensitivity of cardiac muscle contraction increased in M_{21}-TgM**
a) Calcium tension curve of heart muscles from transgenic mice. Relative tension against full tension (%) and calcium concentration (pCa) was plotted for heart muscle fibers from wild type (WT) and high-expression M_{21}-Tg lines 29 and 32.
b) Half maximum pCa for heart muscle fibers.
c) Maximum tension for heart muscle fibers.
d) Schematic representation of cascade for phosphorylation of myosin light chain (MLC). Myosin light chain kinase (MLCK); myosin light chain phosphatase (MLCP); myosin phosphatase target sequence (MYPT).

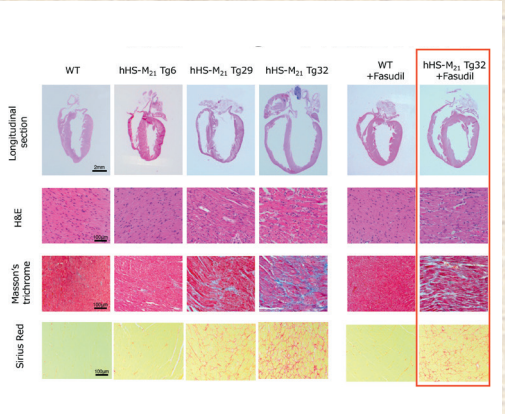

Fig.2: **Rho kinase inhibitor Fasudil prevented heart failure and cardiac fibrosis in M_{21}-TgM**
Pathological findings from the hearts of 8-month-old mice. Macroscopic data of longitudinal section (the most upper field) are shown with microscopic data; hematoxylin eosin (H&E) staining, Manson's trichrome staining and Sirius Red Staining. Wild type mice (WY) and low-expression line (hHS-M_{21} Tg6), high-expression lines (hHS-M_{21} Tg29 and hHS-M_{21} Tg32) were investigated. Data for treatment with a Rho-kinase inhibitor, Fasudil, are shown for wild type mice (WT) and high-expression Tg mice (hHS-M_{21} Tg32).

Systemic Administration of Toll-like Receptor 7 (TLR7) Agonist Enhances the Efficacy of Immune Checkpoint Inhibitors

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RESEARCHERS LED BY TMDU proposed a new combination immunotherapy using a TLR7 agonist to improve treatment efficacy of immune checkpoint inhibitors (ICIs).

The release of negative regulators in immune activation (immune checkpoints) that interferes with beneficial antitumor immune responses brings a benefit to cancer patients. CTLA-4 and PD-1 are such immune checkpoint molecules that negatively regulate T-cell activation. Treatment with humanized antibodies against CTLA-4 and PD-1 (ICIs) have shown to a great achievement in patients with a variety of cancers. Now, immunotherapy has been accepted as the fourth pillar of cancer therapy, following surgery, radiotherapy and chemotherapy. However, patients who receive such benefits of ICIs are limited and differ by their clinical grade and tumor tissue type.

To improve the efficacy of ICI treatment and to reduce economic toxicity by the use of ICIs, the research group has invented a new way to use the synthetic compound of toll-like receptor 7 (TLR7) agonist (resiquimod) as a companion drug of ICIs. TLR7 is a member of the TLR family that recognizes the molecular patterns of various microbes. TLR7-mediated signals lead to the activation of dendritic cells that trigger innate immune responses and subsequently enhances the ability of killer T cells, resulting in the elimination of virally infected cells and tumor cells. Despite such promising effects of resiquimod, its clinical application has been limited in the usage of topical/local application to avoid cytokine storms.

The research group examined the antitumor effects of the systemic application of low-dose resiquimod in two PD-L1 blockade-resistant tumors that exhibited different profiles of tumor-infiltrating lymphocytes (TILs). Resiquimod monotherapy markedly inhibited tumor growth in a squamous cell carcinoma model with abundant infiltration of regulatory T cells (Treg) in the tumor microenvironment. The combinational treatment with PD-L1 blockade further reduced the tumor's growth. Resiquimod monotherapy and combined treatment markedly increased the ratio of CD8 T cell/Treg in the tumor. They found that systemic low-dose resiquimod administration induced earlier activation of two types of dendritic cells (plasmacytoid and conventional), resulting in the reduced recruitment of regulatory T cells and increased recruitment of effector killer T cells in the tumor microenvironment. They further demonstrated that limited doses of resiquimod or decreased frequency of the PD-L1 inhibitor could efficiently regress tumor growth. Their results suggest that limited doses of systemic resiquimod administration enable resistance to PD-1/PD-L1 blockades to be overcome, allowing for the decreased usage of PD-1/PD-L1 inhibitors.

The article, "Systemic administration of a TLR7 agonist attenuates regulatory T cells by dendritic cell modification and overcomes resistance to PD-L1 blockade therapy" was published in Oncotarget (2018, 9:13301, Nishi N et al. at DOI: org/10.18632/oncotarget.24327)

Summary: TMDU researchers developed a new use for TLR7 agonists in cancer immunotherapy. Systemic administration of low-dose resiquimod is useful as a companion drug with PD-1/PD-L1 blockade therapy. This may have great potential to eradicate tumors, especially in immunosuppressive tumors with abundant regulatory T cell infiltration.

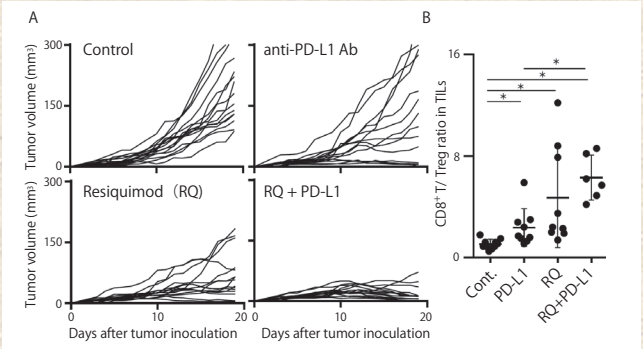


Fig.1: **The effects of treatments with resiquimod and/or PD-L1 blockade in a SCCVII tumor model**
A. Change of tumor volume. Treatments were started on day seven.
B. The ratio of CD8+ T cells/regulatory T cells (Treg) in tumor-infiltrating lymphocytes (TILs) on day 19.

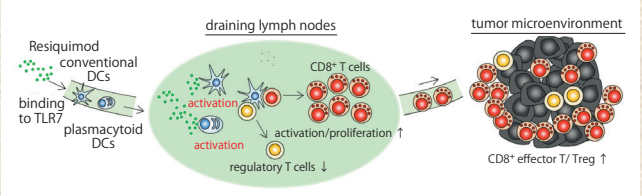
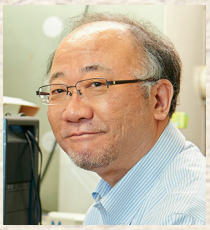


Fig.2: **PPossible mechanism of resiquimod action for enhancing anti-tumor effect**
Systemic administration of low-dose resiquimod induces a transient and rapid activation of plasmacytoid and conventional dendritic cells, resulting in enhanced priming of T cells in draining lymph nodes. Tumor-recruiting CD8⁺ effector T cells increased while regulatory T cells decreased in the tumor microenvironment.

The Intellectual Disability Gene PQBP1 Rescues Alzheimer's Disease Pathology



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TOKYO, JAPAN-ALZHEIMER'S disease (AD) is the most common form of dementia, involving memory loss and a reduction in cognitive abilities. AD is pathologically defined by extracellular beta amyloid (Aβ) aggregates, so therapeutic drugs are being developed with the aim of removing extracellular Aβ aggregates. However, despite the successful decrease in Aβ aggregation, these trials mostly have failed to improve memory or cognitive function in AD patients. This discrepancy highlights the significance of preclinical or prodromal stages of AD.

Now, a Japanese research team led by TMDU has investigated the level of SRRM2 phosphorylation in the AD mouse model and found it to be increased before Aβ aggregation. This ultimately prevented SRRM2 nuclear transport and reduced the level of PQBP1 associated with neurodevelopment and intellectual disorders. The results of the study were reported to Molecular Psychiatry.

In previous work, the research team found that the phosphorylation state of certain proteins changes before the formation of Aβ aggregates in the extracellular space (Tagawa et al., Hum Mol Genet. 2015). Two of the three proteins identified are MARCKS and the homolog MARCKS-like. The third protein was serine / arginine repeat matrix 2 (SRRM 2), thought to be involved in one form of gene regulation, but its precise function has not been elucidated.

"We showed that the increased phosphorylation of SRRM2 prevented it from interacting with another protein which aids protein folding," says first author Hikari Tanaka. "In the absence of this interaction, SRRM2 remained unfolded so it was not transported to the nucleus and was degraded in the cytoplasm." Furthermore, SRRM2 deficiency in neurons destabilized polyglutamine binding protein 1 (PQBP1), a causative gene for intellectual disability (ID), greatly affecting the splicing patterns of synapse-related genes. Actually, the team next measured levels of SRRM2 and PQBP1 protein in the cerebral cortex of early-phase AD mice and human end-stage AD patients as well as in human AD iPS cells. Both proteins were greatly reduced compared with corresponding amounts in healthy controls.

"To find out what effect a reduction in PQBP1 would have *in vivo*, we generated knockout mice in which the PQBP1 gene was disrupted," explains corresponding author Hitoshi Okazawa. "We observed cognitive decline and changes in the morphology of their synapses, which are junctions between neurons that allow electrical and chemical communication. These changes were caused by disrupted patterns of synapse gene splicing."

Restoration of PQBP1 by an adeno-associated virus (AAV) vector was used to recover the synapse protein expression in these mice. Not only did this restore PQBP1 expression, but it also recovered the abnormal phenotypes. This suggests possibilities for gene therapies by virus vectors.

Finally, they identified ERK1/2 (MAPK3/1) as the kinases responsible for the phosphorylation. These results revealed a new aspect of AD pathology: phosphorylation signals that influence RNA splicing and synapse integrity precede extracellular Aβ aggregates and may progress in parallel with tau phosphorylation.

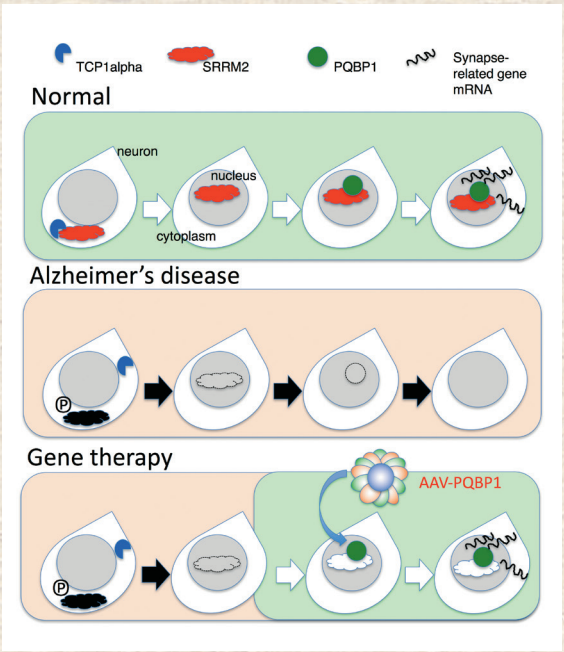

Fig.1: **Theory of Gene Therapy by AAV-PQBP1**
In Alzheimer's state, nuclear scaffold protein SRRM2 and synapse-gene regulator PQBP1 are decreased. By expression of PQBP1 in neurons, expression of synapse genes is recovered, and cognitive defects are rescued in Alzheimer's patients.

Fig.2: **AAV-PQBP1 mediated gene therapy**
By using PQBP1 gene therapy (AAV-PQBP1) in two types of Alzheimer's disease model mice, neural circuit transmission was improved, and memory was recovered even after onset. This suggests a possibility for the same treatment in human patients.